Physiological stress and life-history strategies in the eider (*Somateria mollissima*)

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ACADEMIC DISSERTATION

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To my family

Contents

This thesis is based on the following chapters, which are referred to in the text by their Roman numerals:

Chapter I: Neggazi*, S.A., Noreikiene*, K., Öst, M., & K. Jaatinen. 2016. Reproductive investment is connected to innate immunity in a long-lived animal. *Oecologia* 182: 347–356, doi:10.1007/s00442-016-3657-7

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ABSTRACT

Understanding the causes and consequences of life-history variation is one of the fundamental goals in evolutionary biology. A fairly recent discovery is that even individuals of the same species may differ in their life-histories strategies. However, we still have only a rudimentary understanding of how state and the environment jointly shape such individual life-history strategies within species. Glucocorticoid stress hormones (GCs) may serve as key endocrine modulators integrating information about the internal and external environment. However, we are still lacking detailed knowledge about how GCs may be modulating individually variable life-histories.

In this thesis I set out to explore proximate and ultimate causes of intraspecific variation in life-history strategies, with a particular emphasis on GCs as potential drivers of life-history trade-offs. I have conducted this project on long-lived eider (*Somateria mollissima*) females and their ducklings. Female eiders invest heavily into reproduction which is linked to decreased investment into self-maintenance and survival. Moreover, eiders show repeatable individual differences in stress responsiveness and behavioural strategies, thus serving as a suitable system for studying life-histories in the wild.

The results showed that eiders trade-off self-maintenance for reproduction, but that the magnitude of this trade-off depends on the state (e.g., telomere length, age, body condition) of the individual. The state of the individual is also important in making reproductive decisions in the face of predation risk. In light of my findings, GCs may serve as the link between reproductive investment decisions and reproductive success in the face of danger in eider females. Rather than having uniformly negative effects on reproductive decisions and reproductive success in female eiders, the effects of GCs are context-dependent. The effects of elevated GCs are advantageous in areas of high predation risk by allowing to better prepare for predatory attacks or to shorten incubation time, but may decrease net reproductive output in safer areas. The effects of GCs are also modulated by nest concealment so that females with elevated GCs breed more successfully in open areas with increased perceived predation risk while the opposite is observed in females with low GCs concentrations, having highest reproductive success in well-protected nesting habitats. These result suggest that the contrasting effects GCs may have on fitness are modulated by the environment. Lastly, maternal GCs are important predictors of both stress exposure *in ovo* and early-life telomere length in eider ducklings. The association between maternal GCs depended on offspring sex so that stress exposure increased with maternal GCs in daughters, but decreased in sons.

Overall, the results presented in this thesis highlight the importance of physiological stress in driving life-history decisions. Further, it proposes that GCs may have both beneficial and negative effects on organismal fitness depending on the intrinsic and extrinsic environmental context, be it predation risk or intrinsic environment differences associated with sex of individual. However, GCs are a part of the complex endocrine system and are not acting in isolation. Thus, future studies should address how the concerted interactions between multiple hormones affect life-history decisions in different environmental contexts. Nonetheless, my results suggest that GCs are important elements driving individually variable life-history strategies in the wild. Moreover, the context-dependent association between fitness and GCs levels revealed by this thesis may be part of the mechanism maintaining individual variation in stress responsiveness in the population.

1. INTRODUCTION

Organisms show an astonishing variation in size and age at first reproduction, the total number of offspring produced and in their lifespan. A central tenet of such life-history variation among animals is that everything comes at a cost (Stearns 1992). So, unlike "Darwinian demons", no real organism is able to maximize all fitness components at the same time (Law 1979). Life-histories of organisms are restricted by finitude of resources and other fundamental constraints such as phylogenetic one. This gives rise to a host of trade-offs occurring when investment into one life-history trait leads to a decreased investment into another trait. Trade-offs manifest themselves at different levels: i) at the genetic level as negative genetic correlations; ii) at the physiological level, when at a certain time point resources are divided between competing physiological tasks; iii) at the individual level, where current reproduction is weighted against future reproduction and survival; iv) at the inter-generational level, when for example the optimal level of parental investment differs between the parent and its offspring (Fig. 1). Together, the existence of constraints and fitness trade-offs limits the set of possible life-history traits available to an organism (Roff 1992, Flatt and Heyland 2011).

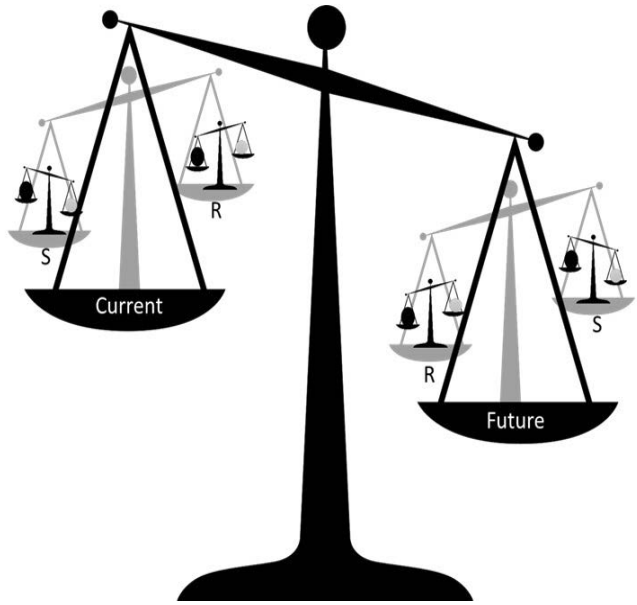


Figure 1. A simplified scheme depicting the organization of life-history trade-offs. Long-lived iteroparous organisms face a trade-off at the individual level between current reproduction and future survival and reproduction (large black balance). Within each time point, there is also a physiological trade-off (grey balances) between how much resources should be divided between competing functions such as self-maintenance (S) and reproduction (R).

1.1. Life-history strategies depend on individual state and the environment

Differences in which life-history traits organisms prioritize give rise to life-history strategies. The variation in investment priorities is obvious at the species level, where species may exist somewhere along the "slow – fast" living continuum (Promislow and Harvey 1990, Williams et al. 2010). "Slow" living species are more likely to invest more into self-maintenance and live longer, while species on the "fast" part of the continuum devote more resources into reproduction which is associated with their shorter life-span (Sæther 1988, Promislow and Harvey 1990). Similar patterns of life-history

differentiation have been also documented between populations (Sinervo et al. 2000, Ghani et al. 2013, DeFaveri et al. 2014). However, there is a growing realization that life-history strategies may also differ between individuals of the same population (Crespi et al. 2013, Schultner et al. 2013). In this case, the key factor influencing optimal resource allocation between survival and reproduction is the state of the individual and its chances of successful reproduction in the future known also as residual reproductive value (McNamara and Houston 1996, Williams 1996). The state of individual is a broad term which includes all features of an organism including physiological, morphological and environmental factors which define its ability to survive and reproduce (McNamara and Houston 1996). Individuals of a semelparous species having just a single reproductive event in their life-time which is followed by a programmed death may prefer to divert the majority of available resources to traits enhancing reproductive success. As a result, inter-individual variation in reproductive success would primarily depend on the efficiency of acquiring, storing and using resources for this one-time reproductive event. In contrast, for individuals of iteroparous species, the optimal strategy of resource allocation may change during their life time. Survival should be a priority over reproduction while individuals are still young and in a good state. However, with decreasing residual reproductive value individuals may prefer to divert proportionally more resources towards reproduction (Pianka and Parker 1975, Forslund and Pärt 1995, Williams 1996). Thus, the reproductive success of iteroparous animals also depends on the ability to optimally distribute resources between several reproductive bouts and self-maintenance.

Life-history decisions are influenced not only by the intrinsic condition of the individual, but also by the environment. When environmental conditions are benign it is easier for animals to acquire resources and the existence of some life-history trade-offs can be masked. This situation is often observed in captive animals which are sheltered from elements and predation and supplied food *ad libitum* (Ricklefs and Cadena 2007, Garratt et al. 2011, but see Uller and Olsson 2005). However, wild organisms are often exposed to challenging environmental conditions. The more challenging the environmental conditions become, the more noticeable life-history trade-offs tend to be (Zera and Harshman 2001). Among the most prominent features of the extrinsic environment are predators. Predation-induced mortality is one of the major causes of death and reproductive failure in nature and, consequently, predators constitute one of the strongest selective forces in nature (Martin 1993, 1995). As a result, animal life-histories are shaped so as to optimize resource allocation by taking into account both the internal state of the animal as well as the selection imposed by predators. For example, reduced reproductive effort at times of high predation risk could be preferred if that would allow to save resources for reproduction later in life when the likelihood of offspring survival and recruitment are better (Zanette et al. 2011). Thus, predation risk provides an incentive for individuals to safeguard their most valuable asset, be it either their current or their future reproduction (Clark 1994).

The diversity of life-history decisions has been of a great interest for centuries (Darwin 1859; Fisher 1930; Stearns 1992, Williams 1996, Crespi et al. 2013). However, we are still struggling to understand what drives life-histories, partly because life-histories are products of complex interactions between intrinsic mechanisms and extrinsic circumstances (Stearns 1992, Roff 1992). This fundamental gap in our understanding may be narrowed by gaining more insight about the proximate mechanisms operating particularly at the level of organismal physiology (Crespi et al. 2013). This is because at this level, complex information from the intrinsic and extrinsic environment of the individual becomes

integrated, potentially acting as a 'driver' predisposing individuals to adopt one or the other life-history strategy.

1.2. Glucocorticoid hormones as mediators of life-history trade-offs

When an animal is facing a challenge, the stress response is a vital mechanism which allows integrating information from internal and external environments, finally serving to return the individual to homeostasis. In vertebrates, most of such reactions involve the hypothalamo-pituitary-adrenal (HPA) axis. Glucocorticoid (GC) hormones are a major group of stress hormones in vertebrates released by the activation of the HPA axis (Sapolsky et al. 2000). In stressful situations, such as when facing predators, GCs coordinate physiological and behavioural responses to the stressor allowing to appropriately react to the stress-inducing stimulus thereby enhancing survival (Sapolsky et al. 2000). Although GCs are often referred to as 'stress' hormones, their functions go beyond reactions to acute stressors. The baseline activity of the HPA axis has a role in regulating metabolism and blood glucose levels, and therefore baseline levels of GCs provide information on the energetic state of the animal (Sapolsky et al. 2000). In contrast to baseline HPA activity and short-term elevations induced by imminent threats, prolonged elevation of GCs can be detrimental. For instance, GCs have been found to have immunosuppressive effects (Keller et al. 1983, Auphan et al. 1995, Bourgeon et al. 2009) which reduce the ability to fight pathogens, and may impair body condition and health in general (Husak and Moore 2008). A prolonged elevation of GC concentrations may act as physiological marker of poor internal state indicating that the individual is challenged by its environment. This view has been embraced widely by the fields of animal welfare and conservation biology (Cockrem 2005, Lane 2006, Cooke et al. 2013). The negative association between GCs and fitness also gave rise to CORT-fitness hypothesis (Bonier et al. 2009b). This hypothesis postulates that individuals with elevated glucocorticoids (corticosterone and cortisol = CORT) have lower reproductive output and survival chances. However, it is important not to forget that elevations in GCs are also essential during important life-history transitions such as in preparation for breeding (McEwen and Wingfield 2003, Denver 2009). It is thus expected that organisms using different life-history strategies should also have different stress-coping abilities (Wingfield and Sapolsky 2003, Monaghan and Haussmann 2015). Therefore, the association between animal fitness and GCs need not be negative in general (Bonier et al. 2009b, Jaatinen et al. 2013). In contrast to captive animals, animals in their natural environment are not shielded from stressors and in fact even chronically elevated GCs can keep on promoting fitness if benefits overweigh the costs (Boonstra 2013). For instance, GCs are also secreted when organisms experience fear (Cockrem and Silverin 2002, Ibáñez-Álamo et al. 2011) which may lead to chronically elevated GCs. However, because GCs can prepare animal for predator attacks and promote survival (Thaker et al. 2010), such elevated GC can still be adaptive. In the following sections, I will review in more detail the association between GCs and traits associated with survival and reproductive success, i.e., components of fitness. Also, I will highlight areas where the lack of knowledge is especially pressing.

The role of glucocorticoids in immunosuppression

The immune system is an important part of the self-maintenance system, defending the animal from pathogens and removing aberrant cells (Jr et al. 2001). The vertebrate immune system can be divided into two branches according to specificity: adaptive immunity which provides slower, but more specific protection from pathogens and innate immunity which acts as an unspecific, but quick first-line of

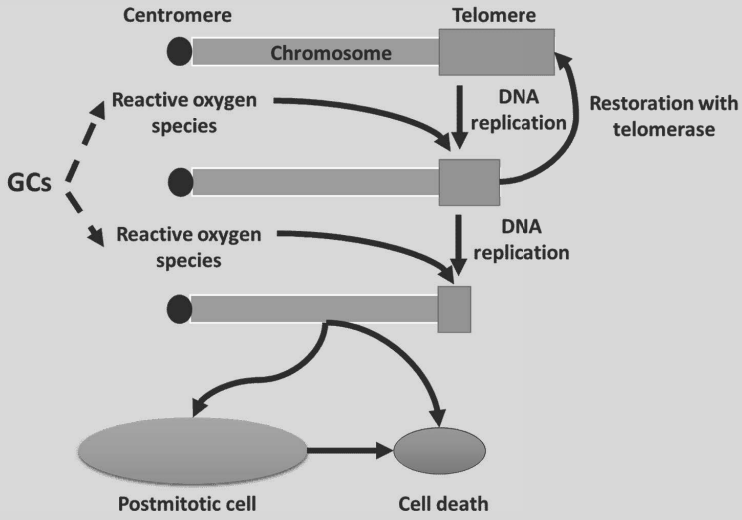
defence against pathogens (Lochmiller and Deerenberg 2000). Both immune systems are affected by stress exposure (Dhabhar 2014). A general notion is that stress in general, and GCs in particular, negatively affect immunity (Auphan et al. 1995, Franchimont 2004, Coutinho and Chapman 2011). Stress-induced immunosuppression can be detrimental leading to higher risk of infections and death (Sheldon and Verhulst 1996). However, more recently, it has been demonstrated that temporary exposure to a stressor may, in fact, enhance immunity (Dhabhar 2000). While in many cases immunosuppression due to chronic stress and prolonged GC exposure have negative fitness consequences, such down-regulation of immune function is not uncommon during certain life-history stages and may be adaptive (Martin et al. 2008). This is because maintaining the immune system at a continuously alert state is costly and decreases energy and nutrients available for important life-history traits such as reproduction (Sheldon and Verhulst 1996, Hasselquist and Nilsson 2012). Although GCs can be elevated during the reproductive period (Romero 2002) which may reduce immunocompetence, this allows to save energy during this energetically demanding period and may thus still represent an adaptive response (Kortet et al. 2003). While suppression of adaptive immunity during reproduction has been reported in a growing number of studies (Ilmonen et al. 2000, Råberg et al. 2000, Cichoń et al. 2001), evidence that innate immunity is suppressed during the reproductive period is still scarce and controversial (Bourgeon et al. 2007, French and Moore 2008, Ruiz et al. 2011). Further, while it is acknowledged that the extent of immunosuppression should depend on both the individual's condition and the external environment (French and Moore 2008, Downs et al. 2015), our understanding of the relative roles of different state attributes in suppressing of innate immunity is still minimal.

Glucocorticoids and telomere shortening

Telomeres are protective nucleoprotein structures capping ends of linear chromosomes and protecting the integrity of the genome (Blackburn 2000). Telomeres shorten with every cell division, which eventually leads to cellular senescence and various associated pathologies (Blasco 2005). The process of telomere attrition can be accelerated by oxidative stress (Von Zglinicki 2000). Oxidative stress describes a condition in which reactive oxygen species (ROS) are not counterbalanced by antioxidants (Sies 1997). The excess ROS causes damage to various cellular structures including proteins, lipids and nucleic acids (Halliwell and Chirico 1993, Cooke et al. 2003, Costa et al. 2007). Because telomeric repeats are high in guanine content they are particularly prone to oxidative damage (Oikawa and Kawanishi 1999) which leads to telomere shortening (von Zglinicki 2002). GCs can elevate oxidative stress (see Box 1) and as a consequence lead to accelerated telomere erosion and more rapid senescence of the organism (Costantini et al. 2011). Therefore telomere length may be indicative of stress exposure and/or stress resistance (von Zglinicki 2002, Epel et al. 2004, Mizutani et al. 2013). So far, telomere length has been linked to individual variation in various phenotypic attributes, including survival (Bakaysa et al. 2007, Haussmann 2010, Monaghan 2010, Heidinger et al. 2012, Angelier et al. 2013). However, the link between telomeres and reproductive decisions is still poorly understood (Beaulieu et al. 2011, Plot et al. 2012, Bauch et al. 2013, Vaillant et al. 2015) and thus, it will be studied in more detail in my thesis.

BOX 1. Telomere length dynamics: a complex process of attrition and restoration of telomeric repeats

Telomeres are conservative structures found in most eukaryotes at the ends of their linear chromosomes. Telomeres consist of short tandem DNA repeats (TTAGGG) and protein complexes. Telomere ends are organized in a t-loop structure that protects telomeres from being recognized as an area of DNA damage. However, during the DNA replication process, an essential step in cell division, a lagging strand of the DNA double helix shortens by the length of the t-loop overhang, also known as DNA-end replication problem. Thus, with every cell division telomeres become shorter. Telomeres get shorter also due to other factors. Glucocorticoids (GCs) and other stressors may increase concentrations of reactive oxygen species and a rich G content in telomeric repeats makes these sequences especially vulnerable to oxidative damage. Telomere length can be restored by the ribonucleoprotein telomerase. However, the activity of this enzyme is often turned-off in tissues to reduce risk of pathologies (e.g. cancer). Thus, if telomere length is not restored and reaches a critical length (ca 400 -1000bp), cells can either die or stay in a postmitotic state. In both cases, accumulation of such cells leads to the process of senescence (aging) which is described as gradual physiological deterioration leading to an increased probability of organismal death.



Preparatory and mediatory roles of glucocorticoids in reproduction

Elevated GC concentrations have conventionally been associated with a negative impact on animal reproduction both in the laboratory and in the field, thus cementing the notion of a generally negative association between GCs and fitness (Smith et al. 1971, Bonier et al. 2009b). For example, in free-living meerkats (*Suricata suricatta*), it has been observed that social stress caused by eviction from the group chronically increases glucocorticoid levels and suppresses the evicted females' reproduction (Young et al. 2006). However, GC levels naturally increase during the reproductive season which may boost reproductive output (reviewed in Romero 2002) and the accumulating literature especially from natural systems indicate that the association between GCs and reproductive effort is far from uniform (Bonier et al. 2009b, 2011, Jaatinen et al. 2013). Thus, these recent findings support the CORT-adaptation hypothesis which suggests that instead of interfering with reproduction, moderately elevated GCs may

facilitate it (Bonier et al. 2009a, 2011, Ebensperger et al. 2011, Crossin et al. 2012). The direction of the association between GCs and reproductive success may also be context-dependent, such that some individuals show positive, some negative, while still others show no relationship between GCs and fitness (e.g., Jaatinen et al. 2013). Given that GCs are linked to reproductive decisions and predation risk (Wingfield and Sapolsky 2003, Sheriff et al. 2011), we may speculate that they play a role as modifiers of reproductive decisions and reproductive success in the face of predation risk. I intend to explore this possibility in my thesis (chapters II and III of this thesis).

Early-life exposure to glucocorticoids shapes the phenotype

Glucocorticoid exposure early in life can have profound effects on the phenotype (e.g. Herborn et al. 2014). Glucocorticoids can be maternally derived when they are transferred through mammalian placentas or deposited in bird or reptile eggs. However, GCs can also be released by activation of HPA axis at later stages of embryonic development and during the early postnatal period (Weinstock 2005). GCs early in life can have: i) hormetic effects, when low concentrations of GCs may have beneficial effects by preparing the individual for the expected environmental conditions; ii) detrimental effects on offspring phenotype; iii) dual effects. Recent studies have showed that effects of early-life stress exposure that may be classified as detrimental are not uncommon (reviewed in Monaghan and Haussmann 2015). A study conducted on long-lived European shags (*Phalacrocorax aristotelis*), for example, found that increased stress exposure of juveniles either due to oral CORT administration or due to handing shortens telomeres (Herborn et al. 2014). Similar results were described for the thorn-tailed rayadito (*Aphrastura spinicauda*), where juvenile birds in an increased brood size treatment had elevated baseline CORT levels which were also negatively associated with their telomere length (Quirici et al. 2016). Interestingly, stress exposure early in life may also have detrimental effects on breeding partners of the exposed individuals (Monaghan et al. 2012). The HPA axis activity is modulated by stressors experienced during development (Marasco et al. 2012) and thereby may prepare individuals for environmental conditions even though this is not cost-free. For instance, European starlings (*Sturnus vulgaris*) that had grown in experimentally increased broods which created a more stressful environment not only had shorter telomeres, but also experienced behavioural changes (Bateson et al. 2015). As adults, individuals raised in enlarged broods had more impulsive feeding behaviour which may be beneficial in a competitive and stressful environments (Bateson et al. 2015). Nonetheless, such preparatory effects could still lead to mostly maladaptive 'thrifty phenotypes' if the parental or offspring early environment are not correlated with the conditions experienced later in life (Barker 1997, Wells 2007, Barrett et al. 2009).

Maternal state and reproductive investment (Fig. 2) are linked to stress experienced by the offspring during development (Mousseau and Fox 1998, Love et al. 2005). In oviparous species, mothers are capable of selectively depositing nutrients and steroid hormones (androgens, glucocorticoids) into the eggs and if females are the heterogametic sex like in birds, then mothers also can bias this distribution according to the sex of their offspring (Young and Badyaev 2004, Love et al. 2005, Badyaev et al. 2006, Sockman et al. 2007). However, the developing embryo is not just a passive receiver of maternally derived hormonal signals. Thus, glucocorticoids deposited by mothers in the eggs can be metabolized by the embryo (Vassallo et al. 2014). Therefore, embryos are capable of modulating the maternal environment. Further, the two sexes differ in their HPA axis activity and sensitivity to GCs

(McCormick et al. 1995, Weinstock 2007, Goel et al. 2014). Two plausible mechanisms for the emergence of such sex-dependent phenotypic differences at least in oviparous species are i) interactions between sex-biased deposition of glucocorticoids and other substances by mothers; ii) sex-specific abilities of embryos to modulate the effects of glucocorticoids. However, up to date, it is unclear how maternal state is related to the overall stress exposure of offspring and whether the effects of maternal state depend on the sex of the progeny.

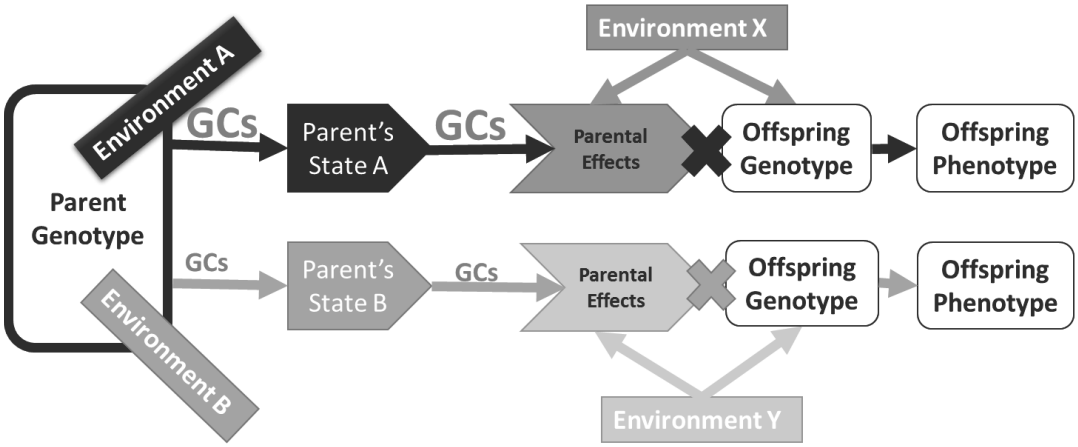


Figure 2. Glucocorticoids (GCs) act as information signals in the early-life environment and are shaping offspring phenotype. The parental environment is interacting with the parent's innate ability to sustain environmental challenges, giving rise to parents which differ in their stress level and state. Such parents may make different investment decisions and thus create early-environments which differ in quality. Variation in the quality of the external and internal early environment may interact with offspring genotype, thus inducing phenotypic plasticity in the offspring.

1.3.Birds as a model system

The interaction between stress responses and their effect on costly physiological functions show promise as determinants of trade-offs between reproduction and survival. Our limited knowledge about physiological constraints on life-history traits mostly come from studies of short-lived, inbred laboratory strains of model organisms guarded from natural conditions (Boonstra 2013). Only studies conducted in natural populations, experiencing variable environmental conditions, will allow us to draw a realistic picture of the physiological stress and life-history variation in the wild. Thus, we are in a dire need of deeper insights into the molecular mechanisms of life-history trade-offs mediated by stress, to fully grasp the evolutionary consequences of varying stress regimes.

The ecology and life-history of birds is extremely well known owing to a large historical interest in this taxon, fuelling recent studies on the physiological mechanisms that may be responsible for such a

diversity of life-history strategies in wild birds (e.g., Haussmann et al. 2005, Angelier et al. 2007, Costantini 2008, Heidinger et al. 2012, Schultner et al. 2013). In contrast to mammals of a comparable size, birds have slower life histories and longer lifespan despite having high metabolic rate, temperature and blood glucose levels, features which are usually associated with a decreased life expectancy (Holmes and Austad 1995, Holmes and Ottinger 2003, Costantini 2008). Further, long-lived birds show slower rate of reproductive senescence and telomere loss than short-lived ones indicating that particularly long-lived species, such as many seabirds, may have evolved special adaptations allowing them to delay senescence (Haussmann et al. 2003, 2005, 2007). Hence, this group is ideal for studies of life-history decisions under varying physiological stress.

2. AIMS

The overall goal of this thesis is to gain insights into proximate and ultimate causes of intraspecific variation in life-history strategies (Fig. 3). More specifically, I aim to disentangle how proximate mechanisms mediate life-history trade-offs in individuals of a long-lived bird species exposed to variable environmental conditions. In chapter **I**, I investigate the trade-off between reproduction and innate immunity. My main prediction was that innate immunity will decrease with increasing reproductive investment. Also that state of individuals will modulate the trade-off. In chapter **II**, I explore how reproductive investment and reproductive success are both linked to the state of individuals as well as to the predation risk they experience. I examine how individual experience and telomere length together with perceived predation risk and varying physiological stress may be associated with variation in clutch size and subsequent hatching success. In chapter **III,** I study the role of nesting habitat in modulating the effects of physiological stress on female reproductive success, with the aim to resolve whether female reproductive success comes at a cost to future survival and reproduction. Lastly, in chapter **IV**, I investigate the transgenerational effects of reproductive investment decisions of individuals of varying state and stress level. In this chapter, my main goal was to disclose how offspring stress experienced *in ovo* and telomere length at hatching are related to maternal state and stress levels. I predicted that these maternal effects may have sex-specific effects on duckling phenotype, because the two sexes differ in their sensitivity to stressors and also may have differential ability to modulate maternal effects.

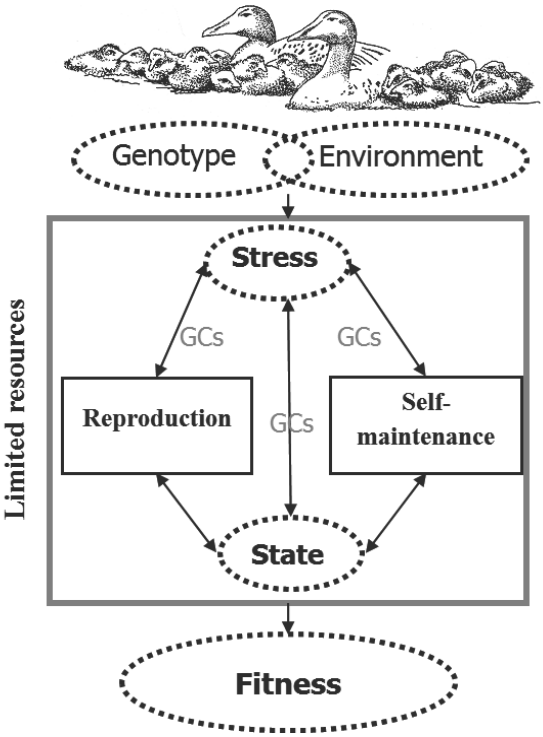


Figure 3. Conceptual outline of the thesis. Physiological stress mediates life-history trade-offs, the expression of which depends on individual quality, ultimately determining fitness. Schematic illustration of an eider brood-rearing coalition modified from Öst et al. (2003).

3. MATERIAL AND METHODS

3.1. Study species

The eider (*Somateria mollissima*) is a large-bodied sea duck. This seabird species is long-lived (up to > 30 years) and widely distributed in the northern hemisphere (Coulson 1984). Females are ground-nesting capital breeders mostly relying on body energy reserves for egg laying and for sustaining incubation (Bolduc and Guillemette 2003, Hobson et al. 2015). They can lose as much as 40% of their pre-incubation weight due to complete incubation anorexia (Parker and Holm 1990). Incubation lasts on average 26 days and during this period, females rely mostly on their camouflage for protection against predators which include both mammals and birds. Ducklings leave the nest within 24 hours after hatching. This suite of life-history characteristics, coupled with uniparental female care (i.e., only females incubate the clutch and care for the young), facilitate the detection of trade-offs between current and future reproduction and result in variable behavioural and physiological responses in females (Öst et al. 2003, Jaatinen et al. 2012, Seltmann et al. 2014).

3.2. Study area

This study was conducted in the archipelago near Tvärminne Zoological Station (59˚50´N, 23˚15´E), in

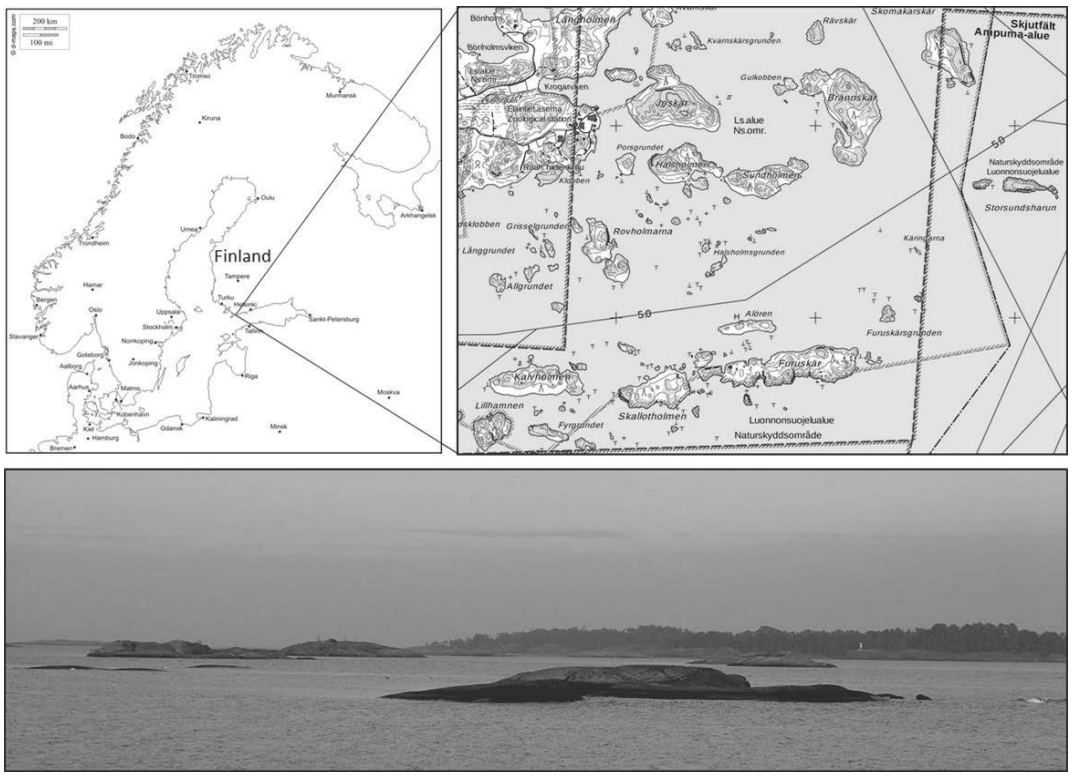


Figure 4. Study area in the archipelago near Tvärminne zoological station in southwestern Finland (upper panel). The study area consists of small rocky islets and bigger islands covered with forest (lower panel).

the western Gulf of Finland. The 23 main study islands (Fig. 4) can either be classified as larger forested islands covered by coniferous and deciduous forest, or smaller islands and islets mainly covered by sparse patches of Juniper scrub (*Juniperus communis*) and grasses ('open islands'). Eider colonies experience a substantial predation pressure during the breeding season. The most common predators preying on adult eiders females are the white-tailed sea eagle (*Haliaeetus albicilla*), the American mink (*Neovison vison*), the raccoon dog (*Nyctereutes procyonoides*), and the eagle owl (*Bubo bubo*). Clutches are most often depredated on by hooded crows (*Corvus corone cornix*), gulls (*Larus* sp.), ravens (*Corvus corax*), American minks and raccoon dogs (Seltmann et al. 2014). White-tailed sea eagles, great black-backed gulls (*Larus marinus*) and herring gulls (*L. argentatus*) pose the greatest threat to ducklings after nest exodus. There is a strong positive correlation between predation pressure experienced by adults and the clutch (1997-2013; r_S = 0.61, N= 17, p = 0.01) (**II**), indicating that measures quantifying annual predation risk are meaningful.

3.3. Field data collection

Females were captured on the nest during the end of the incubation period, during May and early June, using hand nets. Upon capture females were ringed, weighed and measured for structural size as proxied by the length of radius-ulna bone. During the capture, the following set of other variables was recorded: environment (proportional nest cover, island-specific predation risk), reproductive investment (clutch mass, clutch size, incubation stage), female state (body condition, minimum breeding experience). Also, female blood, faeces and feather samples were collected for further laboratory analyses. Nests were re-visited upon hatching. During these visits, female hatching success was recorded based on the number of live ducklings found in the nest. If the ducklings had left the nest prior to the second visit, nesting success was recorded based on the nest contents. Successful hatching is straightforward to determine based on the intact leathery membrane on egg shell remains. In contrast, if eggs were depredated, then typically shattered egg shells with bloody membranes still attached to the shells were found (Öst and Steele 2010). When hatched ducklings were found in the nest, they were weighted and measured for structural size (length of the tarsal bone). A small sample of their blood (<50µl) and 1-2 tail feathers were taken for further analyses.

3.4. Laboratory analyses

DNA extraction

DNA was extracted from either female or duckling blood using the salt extraction method (Aljanabi and Martinez 1997). Extracted DNA underwent rigorous quality control using a spectrophotometer (for concentration and purity) and agarose gel electrophoresis (for integrity).

Telomere assay

Telomere length was measured using the quantitative polymerase chain reaction (qPCR) method (Cawthon 2002). Briefly, the assay includes amplifications of telomeric repeats and a part of a control gene. Any gene which is not variable in copy number such as glyceraldehyde 3-phosphate dehydrogenase gene (*gapdh*) in birds can be used and allow to control for differences in initial quantity of the DNA. The Cq value represents the qPCR cycle number at which the fluorescence reached a fixed

threshold and this value was standardized for interplate variation with GenEx 6 (MultiD) software or by including plate as a random factor in the statistical model. Each reaction plate also included a common standard sample which allowed us to calculate the telomere length of the focal individual relative to that of the standard sample (Pfaffl 2001).

Molecular sexing

Duckling sex (**IV**) was determined by molecular sexing which is based on the amplification of a part of the gene for the chromo-helicase DNA binding protein (Fridolfsson and Ellegren 1999). PCR reaction products were run on an agarose gel, which separates amplicons according to their size. Amplified fragments of female and male eiders were of clearly different size allowing reliable sex determination.

Bacteria killing capacity

The complement protein complex is an essential component of innate immunity acting as a first line of defence against pathogens and it is also one of the major pathways in the development of adaptive immune responses (Janeway et al. 2001, Morgan et al. 2005). Bacteria killing capacity (BKC) of blood plasma estimates an integrated ability of plasma proteins, primarily those belonging to complement, to kill bacteria (Matson et al. 2006, Ricklin et al. 2010, Merrill et al. 2014). The BKC assay was performed using a strain of *Escherichia coli* that is specifically lysed by the complement (Millet et al. 2007, Liebl and Martin II 2009). Briefly, bacteria and diluted plasma samples were mixed and incubated for half an hour during which time bacterial killing occur. Then, a well-mixed bacteria-plasma suspension was planted on Luria Agar plates and incubated overnight to obtain the number of bacteria colonies which are still viable after treatment with the plasma sample (**I**).

Immune cell ratio (H/L ratio)

Immune cell counts and ratios are classic techniques for assessing the state of the immune system and for inferring the state of the individual. One of most widely used measures of avian immune system state is the heterophil to lymphocyte ratio (H/L). It is a good proxy for physiological state because an elevated H/L ratio is linked to recent exposure to physiological stress (Table 1), including, but not limited to, increased reproductive effort (Maxwell 1993, Davis et al. 2008). H/L ratios were estimated from Giemsa stained blood smears by counting the first 100 leucocytes under 1000x magnification (**I**).

Hormone analyses

Quantification of CORT in blood plasma (**IV**), CORT deposited in feathers (fCORT; **IV**) and faecal glucocorticoid metabolites (fGCM; **II** and **III**) were done using radio immune assay (RIA). While all three measures of stress hormone level (baseline CORT, fGCM and fCORT) utilized in this study give information about the concentration of GCs, the biological interpretation of these measures is different (Table 1).

Table 1. Description of different measures of stress in eiders used in this thesis

Stress measure	Function	Description	Reference	Chapter
Heterophil to lymphocyte ratio (H/L)	Physiological state	Correlated with physiological stress experienced during the period of days.	(Davis et al. 2008)	**I**
Baseline plasma corticosterone (CORT)	Energetic state and momentary measure of stress	Measure of circulating GC level in blood (minutes) which was sampled within 3 minutes of eider capture.	(Wingfield and Sapolsky 2003, Bonier et al. 2009b)	**IV**
Faecal glucocorticoid metabolites (fGCM)	Integrated long-term measure of stress exposure	Measure of physiological stress experienced over a period of faeces formation (days-weeks in incubating eiders) which integrates both baseline GC and GCs released during exposure to stressors.	(Möstl et al. 2005)	**II**,**III**
Feather corticosterone (fCORT)	Integrated long-term measure of stress exposure	Measure of stress experienced during the period of feather growth (weeks) which integrates both baseline GC and GCs released during exposure to stressors. In females, the period of feather growth occurs in late summer of the preceding year after the brood-rearing period. In ducklings, the period of feather growth occurs during embryogenesis *in ovo*, a period of ca 26 days in eiders.	(Romero and Fairhurst in press, Bortolotti et al. 2008)	**IV**

3.5. Statistical analyses

In this thesis, I have used linear, generalized linear and linear mixed-effects models. The residuals of models adhered to the assumption of normality. Multicollinearity of predictor variables was assessed by using variance inflation factors; these were always less than 2, indicating that collinearity is not an issue (Kutner et al. 2004, Dormann et al. 2013). Repeatability of H/L scores (**I**) was determined by calculating interclass correlation coefficients (Wolak et al. 2012). All statistical analyses were done using 2.13.0 or 3.0.2 versions of the R software (R core team 2011; 2013) and standard statistical packages compatible with these versions.

4. RESULTS AND DISCUSSION

4.1. Trade-off between immunological state and reproductive investment

Innate immunity is especially important for wild animals, because animals weakened by a prolonged infection can starve or die from predation very quickly (Lochmiller and Deerenberg 2000). However, innate immunity is costly to maintain (McDade et al. 2016), which in times of high energy demands such as during bouts of reproduction may be undesirable. Thus, it may be adaptive to suppress innate immunity specifically during reproduction, even though evidence for this is still both scarce and controversial (Bourgeon et al. 2007, French and Moore 2008, Ruiz et al. 2011). In Chapter **I** of my thesis, I found that innate immune function proxied by blood plasma ability to kill bacteria *in vitro* is negatively associated with clutch size (Fig. 5). This result could be explained by the extreme breeding biology of eiders. Total energy investment into reproduction is especially high in this species, because females depend largely on stored resources for clutch formation (Thomson et al. 1998, Hobson et al. 2015). Further, energetically exhausting egg laying is followed by a demanding female-only incubation period which is aggravated by complete anorexia of the incubating female. Consequently, the efficiency of the blood plasma to kill bacteria is negatively associated with the time spent incubating the clutch (**I**). Taken together, the results of this study suggests that female reproductive investment is traded-off against innate immunity, which represents the first demonstration of such a trade-off in a wild bird. I have investigated only one component of the innate immune system that is based on the ability of complement proteins to kill bacteria. While complement is undoubtedly one of the key components of the innate immune system (Janeway et al. 2001), it is not solely responsible for the functionality of this system. For example, nitric oxide, which is another component of innate immune defence, is not suppressed during the reproductive period in eiders (Bourgeon et al. 2007). Thus, future studies may want to explore whether other components of innate immunity are suppressed in reproducing birds.

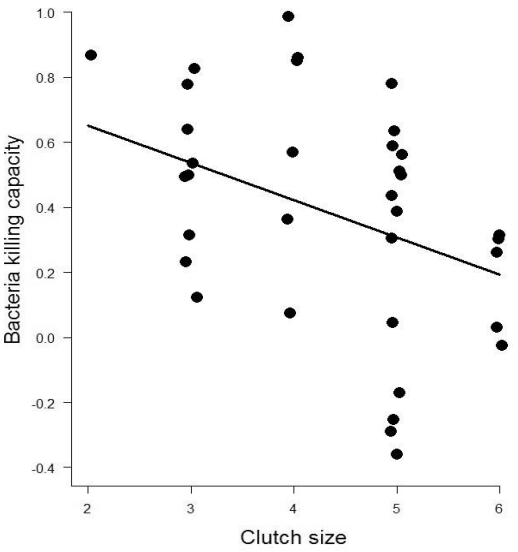


Figure 5. Bacteria killing capacity of blood plasma of incubating eider females is negatively correlated with clutch size.

4.2. Individual state and investment into reproduction

In general, a long-lived animal is expected to prioritize its own survival (self-maintenance) over current reproduction (Stearns 1992). This expectation, however, may be modulated by the state of the individual, such that individuals with low residual reproductive value may achieve higher life-time reproductive success when investing more, rather than less, into current reproduction (Pianka and Parker

1975). The question of how the trade-off between reproductive investment and self-maintenance is resolved in eider females of varying state has been a central question in several chapters of this thesis (**I**, **II**).

In chapter **I,** I describe the association between incubation stage, physiological state (H/L ratio) and investment into innate immunity, as proxied by bacteria killing capacity (BKC) of blood plasma. We hypothesized that only individuals in a good state would have enough resources to decrease their BKC to accommodate the demands of breeding. The results indeed showed that females in better physiological state (lower H/L ratio) decrease BKC as their incubation progresses more than females initially in a poorer state, providing support for the hypothesis. Incubation is as energetically expensive as egg laying in a precocial species with female-only incubation (Deeming and Reynolds 2015). This strain on incubating females is accentuated by the fact that females abstain from feeding during the entire incubation period. Thus, females in a better state are able to save energy by reducing their investment into one of the main innate immunity components –the complement (**I**). Individuals in a poorer state showed a fairly constant level of BKC over the incubation period (**I**), which indicates that they may be close to the lower critical threshold of BKC allowing for adequate pathogen defence. However, an obvious limitation of this cross-sectional study is that it does not allow to control for individual-level changes in BKC over the incubation period. Furthermore, it is unclear whether females in a good state (low H/L) are able to compensate for the loss of BKC with other innate immunity components, or whether the challenges they face (pathogen pressure) is less intense. These open questions would be addressed by adopting a longitudinal approach, in which pathogen pressure and multiple components of the innate system would be simultaneously assessed.

In chapter **II**, I have investigated how clutch size and hatching success are related to female minimum breeding experience and telomere length. In this chapter (**II**), telomere length served as potential indicator of residual reproductive value and phenotypic quality of the bird (Haussmann et al. 2005, Bakaysa et al. 2007, Heidinger et al. 2012, Angelier et al. 2013). It was possible to use telomere length as indicator of residual reproductive value and phenotypic quality because telomere length was uncorrelated to years of breeding experience in adult female eiders studied here (**III**). I predicted that younger females with long telomeres should decrease their current reproductive investment to safeguard resources for future reproduction. Further, I predicted that female hatching success should increase with telomere length, but that breeding experience should positively contribute to breeding success due to an increase in reproductive success associated with experience (Clutton-Brock 1988). The results showed that younger and at the same time less experienced females reduced their reproductive investment with increasing telomere length, while older females increased their clutch size with increasing quality (telomere length) (Fig. 6A). Younger individuals with long telomeres may still have high residual reproductive value which could prove a strong incentive to reduce reproductive investment so as to promote future survival and reproductive prospects (Kotrschal et al. 2007). In line with my predictions, hatching success increased with telomere length (Fig. 6B; **II**), indicating that telomeres may be a molecular marker of quality in eiders. Similarly, an increase in reproductive success with increasing telomere length has been recorded in other long-lived seabirds (Pauliny et al. 2006, Vaillant et al. 2015). Also consistent with my *a priori* expectations, eider female breeding experience was in general positively associated with proportional hatching success (Fig. 6B). Although all individuals with longer telomeres irrespective of age had higher hatching success, older individuals having short telomeres seem able to compensate for their lower phenotypic quality by their breeding experience (Fig. 6B; **II**).

Ultimately, such age-associated improvement in reproductive performance could be explained by the selective disappearance of young, poor-quality individuals (Curio 1983). An alternative proximate explanation could be an age-related increase in the ability to maintain a critical level of prolactin, a

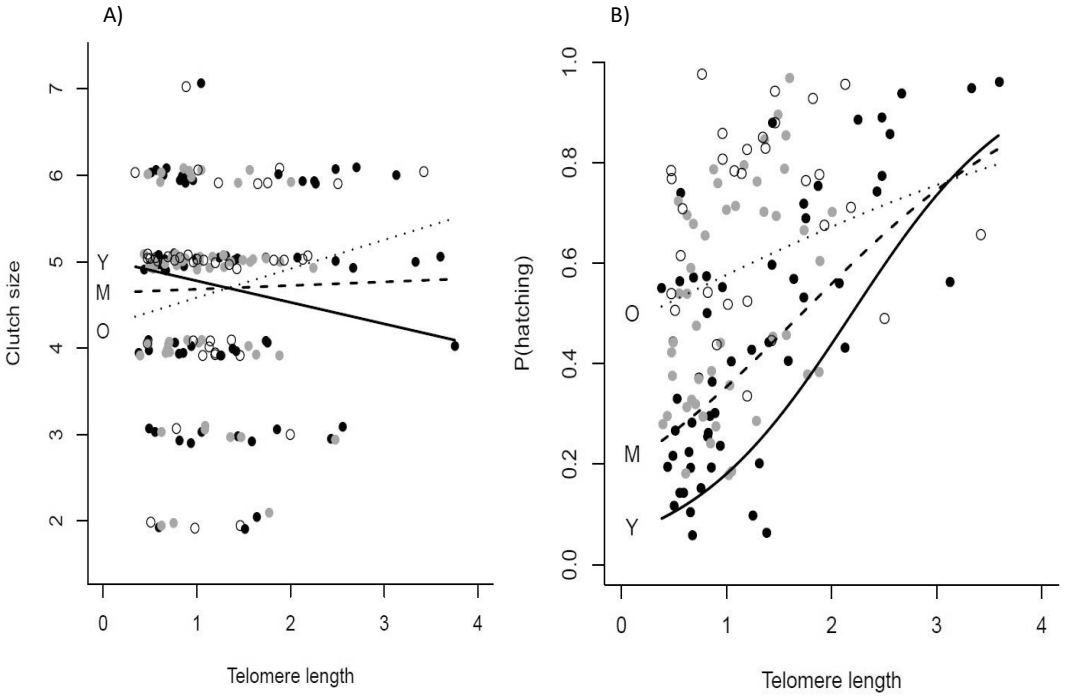


Figure 6. Telomere length and minimum years of breeding experience, a proxy for chronological age, are linked to female clutch size (A) and hatching success (B). For experienced older females (mean + 1 SD, O, dotted line, open circles), clutch sized increases with increasing relative telomere length, and so does their hatching success. In contrast, younger and less experienced females (mean - 1 SD, Y, solid line, black dots) decrease their clutch size within increasing telomere length. Young inexperienced females with the shortest telomeres have the lowest hatching success, but success increases more steeply with telomere length than in other age classes. Intermediate trends are observed for females of an intermediate age (mean, M, dashed line, grey dots). Significant interaction terms are graphically illustrated using simple slope analysis (Aiken and West 1991).

hormone which is essential for parental behaviour (Angelier et al. 2007). I am currently conducting an investigation on the role of prolactin as a potential mediator of reproductive decisions.

4.3. Context-dependent role of glucocorticoids in reproduction

The chances of successful reproduction are reduced at times of high predation, wherefore a reduction in current reproductive investment in order to save resources for future reproduction may be adaptive (Zanette et al. 2011). Considering that GCs have dual effects by facilitating effective responses to predators and predatory cues (Sapolsky et al. 2000, Cockrem and Silverin 2002, Ibáñez-Álamo et al.

2011) and regulating reproductive investment (Wingfield and Sapolsky 2003, Bonier et al. 2009b, Crossin et al. 2012), GCs are prime candidates for modulating reproductive investment in the face of predation risk. To assess the validity of this hypothesis, I investigated how female GC levels and predation risk, measured as the annual island-specific proportion of depredated nests, were related to reproductive investment (clutch size) and proportional hatching success (**II**). Generally, females tended to decrease their reproductive investment under high predation risk with increasing GC levels (Fig. 7A). Result which was consistent with the hypothesized role of GCs in regulating reproductive effort under threat of predation. Furthermore, female fGCM levels were also associated with hatching success under different predation risk (Fig. 7B).

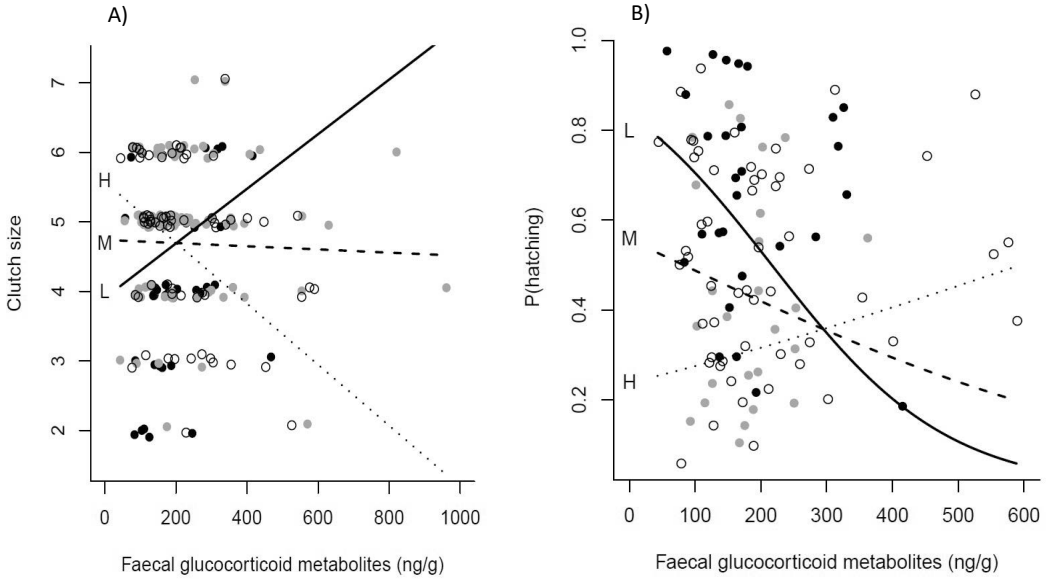


Figure 7. Female faecal glucocorticoid metabolites (fGCM) are linked to both clutch size (A) and hatching success (B) under risk of predation, measured as the annual colony-specific proportion of depredated nests. Under high predation risk (mean + 1 SD, H, dotted line, open circles), clutch size decreases with increasing fGCM, whereas hatching success increases with increasing fGCM. Opposing trends are observed for females nesting in areas of low predation risk (mean - 1 SD, L, solid line, black dots): clutch size increases, while hatching success sharply decreases, with increasing fGCM. Intermediate trends are observed for females nesting in medium risk areas (mean, M, dashed line, grey dots). Significant interaction terms were graphically illustrated using simple slope analysis (Aiken and West 1991).

While hatching success increased with increasing fGCM in females nesting in risky areas, a decreasing trend was observed for females nesting in safer areas. Decreased clutch size in high-risk colonies mediated by fGCM may represent an adaptive response which, according to the 'asset protection principle'(Clark 1994), may allow individuals to channel resources to future reproduction (Zanette et al. 2011). The trend for clutch size to increase with increasing fGCM in safer areas (Fig. 7A) may indicate that moderate increases in fGCMs may be necessary to sustain the increased reproductive effort (Bonier et al. 2011). Interestingly, females subject to high predation pressure having a smaller clutch size (i.e., those with elevated fGCM) nevertheless had higher proportional hatching success. Elevated GC concentrations could prepare individuals for potential threats and thus entail higher hatching success (Romero 2002). Alternatively, elevated GCs may decrease incubation time as was experimentally demonstrated in chickens (Schmidt et al. 2009) and found in eiders (Seltmann et al. 2012) where less bold individuals, the trait which is associated with higher stress-induced GC levels, had shorter incubation period. Such decreased incubation time may be particularly advantageous in terms of hatching success under high predation risk, when each additional day of incubation may greatly increase the risk of nest and female being found by a predator. In contrast, females nesting in safer areas with elevated fGCM had reduced hatching success. This result could be due to the direct negative effects of GCs on hatching success (CORT-fitness hypothesis; Bonier et al. 2009b). Alternatively, but not exclusively, it may stem from a trade-off between egg quantity and quality as females with high fGCM that nested in low-risk colonies had larger clutches (Gustafsson and Sutherland 1988, Smith et al. 1989). These results indicate that increased fGCM could be associated with a higher net reproductive output in areas of high nest predation compared to safer colonies. Thus, the results of this study (**II**) not only support the potential role of GCs in mediating reproductive decisions in the face of danger, but also highlight the context-dependency of fGCM effects.

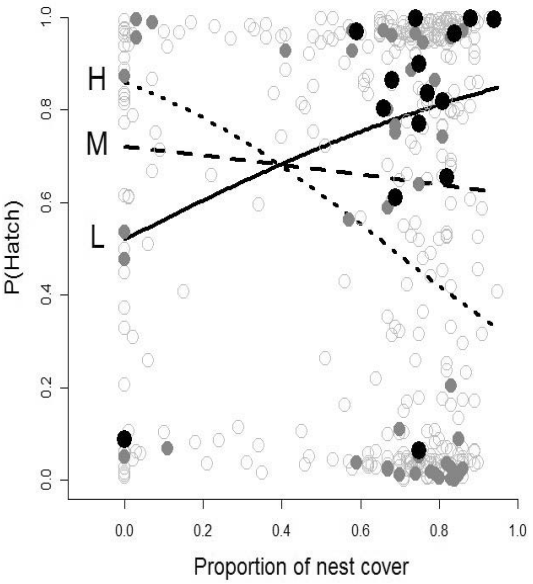


Figure 8. Nest-site cover is linked to hatching success in female eiders, but modulated by the faecal glucocorticoid metabolite (fGCM) level. For females with higher fGCM (mean + 1 SD, H, dotted line, grey dots), proportional hatching success was highest in open nests, but decreased with increasing nest cover. The opposing trend was observed for females with low fGCM levels, having the highest hatching success in well-concealed nests (mean - 1 SD, L, solid line, black dots), but hatching success decreased with decreasing nest cover. Intermediate trends are observed for females nesting in medium risk areas (mean, M, dashed line, open circles). Significant interaction terms were graphically illustrated using simple slope analysis (Aiken and West 1991).

Individuals with higher GC concentrations may be better prepared for breeding in high risk areas in comparison to individuals with lower GC concentrations (Cockrem and Silverin 2002, Romero 2002, Hawlena and Schmitz 2010). This is because GCs are linked to individual's vigilance and escape performance and may prepare individual to react quicker to potential danger (Romero 2002, Chin et al. 2009, Thaker et al. 2010). Nesting habitat and particularly the degree of crypsis provided by nest site is also associated with predator avoidance strategies (Merilaita et al. 1999). Together this suggests that GCs and nesting habitat may be interactively linked to hatching success. Building on this argument and drawing on the findings of chapter **II** (see above), I investigated whether variation in hatching success mediated by fGCM is related to nest-site selection and associated escape strategies (**III**). I hypothesized that the highest proportional hatching success should be achieved when nest sites and GC concentrations are matched, i.e. females choosing nest sites with higher perceived predation risk, usually found in open habitats (Ekroos et al. 2012a), are characterized by a high GC profile, and vice versa (Seltmann et al. 2014). In support of this hypothesis, I found that individuals with higher fGCM levels reached the highest hatching success when breeding in open nests, whereas individuals with lower than average fGCM concentrations had higher breeding success in nest sites offering high concealment (Fig. 8). Further investigation of how this match between fGCM profiles and nest cover may be linked to individual quality (telomere length) revealed that the individuals reaching the highest proportional hatching success paid a cost in terms of shorter telomeres (**III**). Thereby, a coexistence of GC phenotypes in the population may be facilitated by differential nest-site choice and reproductive success under predation-risk (Rivers et al. 2014). It should be noted here that in chapter **II**, predation risk was estimated as island-specific proportion of depredated nests and that both open and concealed nesting sites may be found in every island used in this study. The results of **III** are therefore not a simple replication of those found in **II** (Fig. 7) as island identity and nest-site cover are not intercorrelated.

Taken together, the results of chapters **II** and **III** support the idea that GCs have an important role in modulating reproductive decisions in the presence of predators and that the effects of GCs on hatching success are context specific (**II**) and potentially mediated by different escape strategies of prey (**III**). In the future, it would be interesting to investigate how the interactions between predation risk, nesting habitat and fGCM levels ultimately affect survival and lifetime reproductive success.

4.4. Maternal stress and state predict sex-specific early-life telomere length and stress exposure

Exposure to glucocorticoids during development and early in life can shape the phenotype in both hormetic and detrimental ways (Chin et al. 2009, Haussmann et al. 2012, Herborn et al. 2014, Monaghan and Haussmann 2015). Key factors influencing offspring exposure to glucocorticoids are maternal state, maternal reproductive investment (Mousseau and Fox 1998, Love et al. 2005) and the innate ability of developing embryos to modulate such maternal effects (Vassallo et al. 2014).

In the last chapter of my thesis (**IV**), I analysed how telomere length and feather corticosterone (fCORT) of newly-hatched ducklings is related to maternal stress (proxied by baseline CORT and fCORT) and state (breeding experience, telomere length and body condition). I found that duckling stress exposure was associated with maternal telomere length in a sex-specific manner, such that stress exposure increased with maternal telomere length only in female offspring. Further, I found that maternal stress proxied by fCORT had sex-specific effects on both duckling telomere length (Fig. 9A) and their fCORT (Fig. 9B). Telomere length (Fig. 9A) decreased with increasing maternal fCORT in

males, but showed an opposite trend in females. Because early-life telomere length may predict survival prospects (Heidinger et al. 2012), male offspring of high fCORT mothers may have a reduced life expectancy. Interestingly, male offspring and maternal fCORT were negatively associated, while the opposite trend was observed in female offspring (Fig. 9B). Thus these results are not in line with the expectation that telomere length is invariably reduced due to higher stress exposure (Herborn et al. 2014), but instead indicate a sex-specific response to maternally-induced stress. One possible explanation for such result is that males and females differ in their sensitivity to stressors (Weinstock 2007, Barrett and Richardson 2011). Such sex-biased effects may stem not only from differences in sensitivity to glucocorticoids *in ovo*, but may also involve other steroid hormones. Sex hormones are known to be deposited by mothers into eggs in a biased way according to the sex of the offspring, thus creating different offspring phenotypes in the same brood (Groothuis et al. 2005, Eising et al. 2006). While glucocorticoid exposure increases oxidative stress and is associated with reduced telomere length (Costantini et al. 2011, Herborn et al. 2014), other steroid hormones can have additive effects or effects

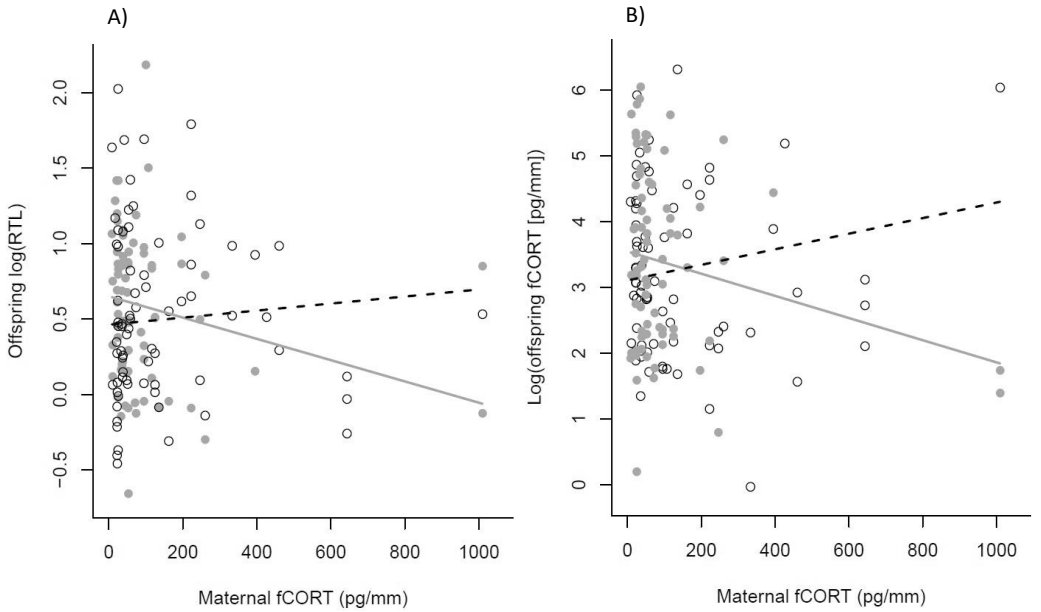


Figure 9. Sex-specific effects of maternal feather corticosterone (fCORT) on offspring relative telomere length (RTL; A) and feather corticosterone (B). With increasing maternal fCORT, the telomere length and fCORT of daughters increase (dashed line, open circles). The opposite trend is observed in sons, in which both telomere length and feather CORT decrease with increasing maternal fCORT. Significant interaction terms were graphically illustrated using simple slope analysis (Aiken and West 1991).

that outweigh the effects of glucocorticoids, thus interactively shaping the early-phenotype of the duckling. On the one hand, oestrogen can have beneficial effects for offspring telomeres as this hormone can activate telomerase which restores telomere length (Behl et al. 1997, Kyo et al. 1999, Bayne et al. 2008). On the other hand, however, testosterone can lead to elevated oxidative stress (Alonso-Alvarez et al. 2007) which may negatively affect telomere length.

Overall, the results revealed the presence of several maternal factors contributing to early-life variation in telomere length and stress exposure *in ovo* (**IV**), thus addressing the recent call for studies elucidating how maternal effects caused by environmental conditions encountered by the reproducing female contribute to offspring phenotype (Asghar et al. 2015, Monaghan and Haussmann 2015). However, several open questions remain that would merit future research attention (**IV**). First, it would be imperative to assess the role of other steroid hormones in shaping offspring phenotype in a sex-specific manner and to investigate whether offspring fCORT can actually predict duckling behaviour and survival later in life. Further, it is not clear why female fCORT rather than baseline plasma CORT was associated with duckling stress exposure in the egg. Possibly this may be explained by the fact that baseline plasma CORT reflects the momentary levels of circulating CORT which also can change rapidly thus presenting only a small window for discerning the activity of the HPA axis. Consequently, plasma CORT may not be an accurate predictor of the realistic exposure to physiologically relevant stimuli (Fairhurst et al. 2013). However, to address this question in more detail in the future it would be important to obtain a better understanding of the temporal variation in predation risk and how this is reflected in maternal fCORT.

5. CONCLUSIONS AND FUTURE DIRECTIONS

In this thesis, I have investigated proximate mechanisms that mediate individually variable life-history strategies in eider ducks. The main finding is that life-history decisions depend on the state of the individual and the environmental context individuals are faced, with glucocorticoid stress hormones playing a major role as a mediator between competing demands. Further, the results highlight the importance of context in determining the sign of the association between glucocorticoids and fitness and clearly contradict the notion of a uniformly negative association between the two (Bonier et al. 2009b). Ultimately, the results suggest that the context-dependent effects of glucocorticoids may actually contribute to maintaining variable glucocorticoid phenotypes in the population. This is because at the end, neither high nor low glucocorticoid phenotypes are invariably 'good' or 'bad' from an evolutionary standpoint, as this all depends on the environment faced by the animal, as well as on its internal state.

To summarize the main findings, female eiders trade-off innate immunity, measured as the complement's ability to kill bacteria, against reproduction. This is one of the first evidence of such associations observed in the wild (**I**). However, also in this case, the magnitude of the trade-off between immunity and reproductive investment depends on the state of the individual (**I**). Individual state was also an important factor influencing reproductive investment and success in the face of predation (**II**). Thus, the clutch size of females of a variable state, estimated by their telomere length, body condition and breeding experience (age), varied in a manner reflecting the females' need to protect their greatest reproductive asset, be it either current of future reproductive investment (Clark 1994). Chapter **II** highlights how female state variables (telomere length, breeding experience) interactively explained not only reproductive investment, but also hatching success. While females of a higher phenotypic quality proxied by telomere length had higher hatching success, older and more experienced females may be

able to compensate for their poorer intrinsic quality by their greater breeding experience (**II**). Maternal telomere length was also associated with duckling stress exposure *in ovo* so that stress exposure increased with increasing maternal telomere length in female offspring, but not in male offspring (**IV**).

These results provide evidence that glucocorticoids may indeed serve as the link between reproductive investment decisions and reproductive success in the face of danger in eider females (**II, III**). Rather than having a generally negative impact on fitness, glucocorticoids show context-dependent associations with eider female reproductive decisions and reproductive success. In general, the effects of elevated glucocorticoids are advantageous in areas of high actual (**II**) or perceived (**III**) predation risk, whereas elevated glucocorticoids may be associated with reduced net reproductive output in safer areas. These results suggest that the contrasting effects glucocorticoids may have on fitness are modulated by the environment. Lastly, maternal glucocorticoids are important predictors of both stress exposure *in ovo* and early-life telomere length in eider ducklings (**IV**). The association with maternal glucocorticoids was, however, contrasting between the two offspring sexes.

There are several potentially fruitful avenues to further refine our understanding of the association between glucocorticoids and life-history strategies. One such avenue would be to conduct a longitudinal study where the same individuals are followed throughout their life to assess the lifetime fitness of individuals having variable life-history strategies. This would help to understand if, for instance, down-regulation of the innate immune system which is beneficial by increasing current reproductive investment (**I**) has similar effects in different years and, at the end, if safe-guarding self-maintenance versus reproductive investment does help to improve or optimize life-time reproductive success. Further, a longitudinal approach will help to understand how the observed match between nesting habitat and glucocorticoid profiles, also related to hatching success and telomere length (**III**) could be related to the survival of eider females that 'match' their stress profile with the nesting habitat and have higher hatching success, but shorter telomeres, as opposed to those females that apparently 'fail' in this matching. Such a comparison will ultimately be possible once more years of data of the females in the Tvärminne study population become available.

Another line of research that I particularly would encourage to pursue would be to investigate in more detail the effects of maternal stress described in **IV**. Unfortunately, the longitudinal nature of the research conducted in this eider population and the alarming population trend of eiders in northern Europe (Ekroos et al. 2012b, BirdLife International 2015) do not permit experimental manipulations or extensive disturbances of breeding birds. However, the collection of longitudinal data and usage of modern molecular techniques would still allow inspecting the sex-dependent association between maternal fCORT and duckling telomeres and fCORT in greater detail. For example, it would be interesting to assess if the level of antioxidants and oxidative stress in eider ducklings are also associated with maternal stress, perhaps showing a sex-biased pattern, and whether this, in turn, could explain differences in telomere length. A complementary analysis may involve analyses of sex hormones and telomerase expression or activity, as that could further our understanding of the role of sex hormones in explaining the observed sex-dependent association between stress *in ovo* and telomere length.

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